



Europäisches Patentamt
European Patent Office
Office européen des brevets



Publication number:

0 412 557 A1

(1)

EUROPEAN PATENT APPLICATION

(2) Application number: 90115397.3

(3) Date of filing: 10.08.90

(5) Int. Cl.⁵ C07K 13.00, C12N 15.16,
C12P 21.02, C12N 5.10,
A61K 37.24

(8) Priority: 11.08.89 JP 209449/89
03.04.90 JP 88592/90
27.07.90 JP 200898/90

(43) Date of publication of application:
13.02.91 Bulletin 91/07

(34) Designated Contracting States:
AT CH DE FR GB IT LI LU NL SE

(71) Applicant: MITSUBISHI KASEI CORPORATION
5-2, Marunouchi 2-chome Chiyoda-ku
Tokyo 100(JP)

(72) Inventor: Kitamura, Naomi, 910
Merodii-Haimu-Dainichi
2-272 Yakumo-Higashi-machi
Moriguchi-shi, Osaka-fu(JP)
Inventor: Miyazawa, Keiji
6-13, Korien-cho
Hirakata-shi, Osaka-fu(JP)
Inventor: Daikuhara, Yasushi
4-14-10-41 Meiwa
Kagoshima-shi, Kagoshima-ken(JP)
Inventor: Tsubouchi, Hirohito

1925 Harara-cho
Kagoshima-shi, Kagoshima-ken(JP)
Inventor: Naka, Daiji
23-4, Tana-cho, Midori-ku
Yokohama-shi, Kanagawa-ken(JP)
Inventor: Takahashi, Kazuhiro
2-10-1, Naruse
Machida-shi, Tokyo(JP)
Inventor: Matsui, Rie
2-24-6-101, Nishikata
Bunkyo-ku, Tokyo(JP)
Inventor: Yoshiyama, Yoshiko
6-20-41, Satsukigaoka, Midori-ku
Yokohama-shi, Kanagawa-ken(JP)
Inventor: Ishii, Takehisa
1-21-6-612, Matsumi-cho, Kanagawa-ku
Yokohama-shi, Kanagawa-ken(JP)

(74) Representative: Kolb, Helga, Dr. Dipl.-Chem. et
al
Hoffmann, Eitle & Partner Patentanwälte
Arabellastrasse 4
D-8000 München 81(DE)

(54) Hepatic parenchymal cell growth factor, gene encoding the same, process for producing the factor, and transformants producing the factor.

(57) According to the present invention, there are provided hepatic parenchymal cell growth factor obtained by recombinant DNA technology, a gene coding for the factor, an expression vector capable of expressing the gene, a cell, in particular animal cell, transformed with the expression vector, and a process for producing the hepatic parenchymal cell growth factor.

EP 0 412 557 A1

HEPATIC PARENCHYMAL CELL GROWTH FACTOR, GENE ENCODING THE SAME, PROCESS FOR PRODUCING THE FACTOR, AND TRANSFORMANTS PRODUCING THE FACTOR

BACKGROUND OF THE INVENTION

5 Field of the Invention

This invention relates to a hepatic parenchymal cell growth factor obtained by the recombinant DNA technology; to a gene encoding it; to a transformant carrying an expression vector which comprises at least a promoter sequence required for the expression of a protein, a sequence encoding a signal peptide, a DNA sequence encoding human hepatic parenchymal cell growth factor, and a terminator sequence; and to a process for the production of human hepatic parenchymal cell growth factor by culturing the transformant.

Description of the Prior Art

15 The liver is the most highly differentiated and largest adenogenous organ in a living body. It exhibits various important functions such as treatment (metabolism), storage, detoxication, decomposition, excretion and the like of various nutritive substances (carbohydrates, proteins, lipids, vitamins, hormones and the like), and especially it plays an important role in the intermediate metabolism of a living body.

20 These functions are sustained by hepatic parenchymal cells which are controlled by various hormones in a living body and may show remarkably active proliferation in certain cases. In a rat, for example, it has been known that even after surgical resection of about two-thirds of the liver the remaining hepatic tissue promptly grows and may be restored to its original size in about 10 days. On the other hand, patients suffering from hepatic carcinoma have been treated by partial hepatectomy followed by regeneration.

25 A large number of researches and investigations have been pursued to elucidate the mechanism of hepatic regeneration by the proliferation of hepatic parenchymal cells, with reports suggesting the presence of hepatic parenchymal cell growth factor. Especially, some of the present inventors found that plasma from patients with fulminant hepatitis had a markedly high activity to proliferate hepatic parenchymal cells (*Biomed. Res.*, 6, 231 (1985) and *Exp. Cell Res.*, 166, 139 (1986)) and succeeded for the first time in the world in purifying the proliferation-activating factor as a single protein (Japanese Patent Application Kokai No. 22526/1988 and *J. Clin. Invest.*, 81, 414 (1988)).

30 This human hepatic parenchymal cell growth factor (human hepatocyte growth factor; to be referred to as "hHGF" hereinafter) had a molecular weight of approximately 76,000 to 92,000 as estimated by SDS-PAGE under non-reducing conditions, but SDS-PAGE under reducing conditions revealed two bands at molecular weights of 56,000 to 65,000 and 32,000 to 35,000. Nakamura *et al.* reported rat platelet derived factor having similar activity (*Biochem. Biophys. Res. Commun.*, 122, 1450 (1984)), and estimated its molecular weight to be approximately 27,000 by SDS-PAGE (*Proc. Natl. Acad. Sci. USA*, 83, 6489 (1986)). Thereafter, they purified the factor as a homogeneous protein and reported that the purified factor was a protein having a molecular weight of 82,000, which consisted of two polypeptides having molecular weights of 69,000 and 34,000 (*FEBS Letters*, 224, 311 (1987)).

40 Except for the above mentioned hHGF and rat HGF, there has been no report on any hepatocyte growth factor which has been purified as a homogeneous protein. Even with regard to the hHGF and rat HGF, we know of no report concerning their primary structures and corresponding cDNA base sequences.

45 A large amount of hHGF will be required when an examination is to be performed in order to elucidate the function of hHGF in a living body in detail and/or its effects on the hepatic regeneration in a patient with hepatopathy. However, isolation and purification of a large amount of hHGF from plasma of patients with fulminant hepatitis are not so easy in view of labor, time and economy, and stable isolation of only hHGF from sera in which various infectious agents exist is extremely difficult to achieve. Because of these reasons, stable and large scale isolation and purification of hHGF from plasma of patients with fulminant hepatitis have not been attempted.

SUMMARY OF THE INVENTION

The present inventors have conducted intensive studies for the purpose of obtaining a large amount of hHGF by the recombinant DNA technology and succeeded for the first time in cloning a gene coding for hHGF, which is useful for such a purpose. Further, the present inventors have constructed a new expression vector containing the gene, enabling the expression of hHGF. Thus, the present invention has been attained.

Accordingly, it is an object of the present invention to provide a hepatic parenchymal cell growth factor obtained by the genetic engineering.

Another object is to provide a gene coding for such a hepatic parenchymal cell growth factor.

Still another object is to provide an expression vector containing the gene.

A further object of the present invention is to provide a transformant, for example, an animal cell, carrying the expression vector, which is capable of producing the hepatic parenchymal cell growth factor.

A still further object is to provide a process for producing the hepatic parenchymal cell growth factor by the recombinant DNA technology.

The other objects of the present invention will be apparent from the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood by the following description with reference to the attached drawings in which:

Fig. 1 a to d show the amino acid sequence of the hHGF of the present invention;

Fig. 2 a to c show the base sequence of the cDNA obtained in Example 1, which contains a gene coding for the hHGF of the present invention. Recognition sites of principal restriction enzymes are also shown in this figure. Underlines in the figure indicate the regions which correspond to the amino acid sequences already determined, and, of these, double underlines indicate the sequences that correspond to the probe used in the first cloning according to the present invention;

Fig. 3 shows a scheme for the construction of a vector capable of expressing human parenchymal cell growth factor;

Fig. 4 shows the structure of an expression vector containing the DNA coding for the human parenchymal cell growth factor according to the present invention; and

Fig. 5 is a graph showing the biological activity of the supernatant containing human parenchymal cell growth factor produced by CHO cells carrying the expression vector which has the DNA coding for the human parenchymal cell growth factor of the present invention.

DESCRIPTION OF THE INVENTION

According to the present invention, there are provided human hepatocyte growth factor (hHGF) represented by the amino acid sequence as shown in Fig. 1, which contains a signal sequence; hHGF represented by the amino acid sequence ranging from the 30th glutamic acid residue (Glu) to the last serine residue (Ser) in Fig. 1; and hHGF represented by the amino acid sequence ranging from the 32nd glutamine residue (Gln) to the last serine residue (Ser) in Fig. 1. The present invention also comprises modifications of hHGF as mentioned above which may have at least one addition, deletion or replacement of an amino acid, provided that these modifications are functionally equivalent to naturally occurring hHGF.

Also provided are a gene coding for the hHGF represented by any of said amino acid sequences; the gene represented by the base sequence as shown in Fig. 2, which codes for hHGF containing a signal sequence;

the gene represented by the base sequence ranging from the 88th base, guanine (G), to the last base (G) in Fig. 2; and the gene represented by the base sequence ranging from the 94th base, cytosine (C), to the last base (G) in Fig. 2. The present invention also provides a process for producing hHGF represented by the amino acid sequence of Fig. 1 which comprises transforming a host cell with an expression vector containing a gene coding for the hHGF, and culturing the resulting transformant. Further, a transformant capable of producing the hHGF is provided according to the present invention.

The gene (cDNA) coding for the hHGF of the present invention may have the base sequence as shown in Fig. 2, in which, however, only the base sequence of a single-stranded DNA is described while complementary base sequences are omitted as a matter of convenience.

The gene may be used to express hHGF having the amino acid sequence as shown in Fig. 1 by the

recombinant DNA technology. In that case, a protein translated from the corresponding mRNA coding for the hHGF contains a signal sequence. This signal sequence will be cleaved off when the protein is secreted from host cells, thus resulting in the production of hHGF having the amino acid sequence which ranges from the 30th glutamic acid residue (Glu) or the 32nd glutamine residue (Gln) to the last amino acid residue of the sequence represented by Fig. 1. Instead of this signal sequence, certain signal sequences of other proteins may also be used herein. On the other hand, when mature hHGF having no signal sequence is to be expressed in host cells, an hHGF-encoding gene having the base sequence which ranges from the 68th G or the 94th C to the last base of the sequence represented by Fig. 2, may be used after ligating the gene with an ATG codon of a vector DNA.

In accordance with the present invention, it is intended to include all modifications such as elimination, change, and addition of one or more amino acids or nucleic acids, provided that the growth-enhancing activity of the hepatic parenchymal cells is not altered.

A DNA fragment of a gene coding for hHGF of the present invention may be obtained by the following procedures:

According to the method described in J. Clin. Invest., 81, 414 (1988), hHGF may be purified from plasma of patients with fulminant hepatitis. The purified hHGF will be dissociated into two polypeptides by the breakage of disulfide bonds under reducing conditions. The larger polypeptide having a molecular weight of 56,000 to 65,000 is called "H chain", and the smaller one having a molecular weight of 32,000 to 35,000 "L chain".

The purified hHGF is reduced and the thiol groups of cystein residues formed are carboxymethylated followed by subjecting to reverse-phase high-pressure liquid chromatography to isolate the H and L chains. Alternatively, hHGF may be electrophoresed under a reducing condition on a gel, from which each of the H and L chains is extracted. The N-terminal amino acid sequences of the both chains may then be determined by analyzing the chains by Applied Biosystems gas phase protein sequencer.

On the other hand, hHGF itself, or after separating it into H and L chains, may be hydrolyzed by an appropriate proteolytic enzyme, such as *Achromobacter* Protease I (lysyl endopeptidase). The resulting peptide fragments may be isolated by reverse-phase high-pressure liquid chromatography. Each peptide may be analyzed as described above to determine an internal amino acid sequence of the polypeptide.

From these amino acid sequences, DNA base sequences may be deduced to select a sequence suitable for the preparation of an oligonucleotide, for example, the one as shown in Examples described hereinbelow. Such an suitable oligonucleotide is synthesized and used as a probe.

A cDNA library to be screened for a gene coding for hHGF may be any one derived from human liver, spleen or placenta and is commercially available from Clontech Laboratories, Inc. A placental cDNA library is particularly preferred. Further, cDNA library may also be prepared in a usual manner from a cell line or tissue material in which hHGF is expressed.

E. coli is infected with lambda phage which contains such a cDNA and cultured, in accordance with the method of Maniatis (Molecular Cloning, A Laboratory Manual, pages 56 to 73, Cold Spring Harbor Laboratories, 1982). Plaques thus formed are then subjected to a selection process using as a probe an oligonucleotide prepared above based on the base sequence deduced from a portion of the amino acid sequence of hHGF, according to the plaque hybridization method (Molecular Cloning, A Laboratory Manual, pages 320 to 328, Cold Spring Harbor Laboratory, 1982). Thus, several different lambda phage clones may be easily obtained, each clone having both the base sequence used as the probe and a base sequence corresponding to the other region of the amino acid sequence of the desired hHGF.

The positive plaques in the screening process are selected and the phages are grown according to the method of Maniatis (Molecular Cloning, A Laboratory Manual, pages 76 to 79, Cold Spring Harbor Laboratory, 1982). DNA is purified by the glycerol gradient method and digested with an appropriate enzyme, such as *Eco* RI. The resulting cDNA is then subcloned into a plasmid vector, such as pUC18 and pUC19, or a single stranded phage, such as M13mp18 and M13mp19. The base sequence of a desired cDNA segment may be determined according to the dideoxy chain termination method of Sanger et al. (Proc. Natl. Acad. Sci. USA, 74, 5463 (1977)). In this manner, the obtained cDNA sub-clones are analyzed to determine the base sequences, which may code for different portions of hHGF and be combined (Fig. 2). Thus, the whole base sequence of a gene coding for the entire amino acid sequence of hHGF as shown in Fig. 1 may be determined.

Industrial production of hHGF will require the selection of a host-vector system capable of providing stable expression. Also, expressed hHGF must possess the biological activity to proliferate hepatic parenchymal cells. In particular, it should be taken into consideration that naturally occurring hHGF is a glycoprotein, that hHGF contains a number of cyteine residues, and that the positions of disulfide bonds formed between thiol groups in the cysteine residues and the higher order structure play an important role

in maintaining the activity of hHGF.

Taking the above into consideration, it is desirable to use animal cells, for example, CHO, COS, and mouse L, C127 and FM3A cells, as host cells for expressing the hHGF gene according to the present invention, although microorganisms, such as yeast, eg., *Saccharomyces cerevisiae*, and *Escherichia coli*, eg., *E. coli* YA-21, may be used. Further, when such an animal cell is used as a host cell, it will be advantageous that a gene coding for immature hHGF containing a signal sequence, i.e., such a gene that also contains 1 to 37, or 1 to 93 nucleotides of the DNA sequence as shown in Fig. 2, is used and incorporated into the cell, since the mature hHGF protein is expected to be secreted in culture media.

An expression vector which may be used in the present invention contains a DNA fragment coding for at least a portion of the amino acid sequence of the hHGF protein downstream from the promoter of the vector. It may be contemplated herein to use various promoters, including SV40 promoter, the promoters of apolipoprotein E and A1 genes, of heat shock protein gene, and of metallothionein gene, HSV TK promoter, adenovirus promoter, and retrovirus LTR. In the present invention, however, SV40 promoter or the promoter of metallothionein gene is preferred.

A DNA fragment coding for immature hHGF containing a signal sequence is inserted into a vector downstream from its promoter in the direction of transcription. It is possible to insert a combination of two or three such hHGF DNA fragments. Also, it may be possible to prepare such a unit that comprises an hHGF DNA fragment, 5' upstream of which a promoter is linked to, and to insert two or three such units into a vector in tandem along the direction of transcription.

A polyadenylation signal should be present downstream from the hHGF gene in the expression vector. Such a polyadenylation signal may be derived from SV40 DNA, beta-globin gene, or metallothionein gene. When two or three DNA fragments comprising a promoter linked to the hHGF gene as described above are inserted in tandem into a vector, it is possible to link a polyadenylation signal to 3' of each hHGF gene.

It is desirable to use a selective marker when an animal cell such as CHO cell is transformed with the expression vector. Such a selective marker gene may be inserted into the expression vector downstream from the polyadenylation signal along or against the direction of transcription, otherwise another plasmid containing a selective marker gene must be co-transformed to obtain a transformant. Such selective markers may include DHFR gene providing methotrexate resistance (J. Mol. Biol., 159, 601 (1982)); Neo gene providing G-418 antibiotic resistance (J. Mol. Appl. Genet., 1, 327 (1982)); *E. coli* derived Ecogpt gene providing mycophenolic acid resistance (Proc. Natl. Acad. Sci. USA, 78, 2072 (1981)); and hph gene providing hygromycin antibiotic resistance (Mol. Cell. Biol., 5, 410 (1985)). The selective marker gene has a promoter, for example, SV40 promoter, 5' upstream thereof and a polyadenylation signal 3' downstream therefrom.

As already described, when such a selective marker gene is not inserted into the expression vector, another vector or plasmid which contains a marker enabling the selection of a transformant may be co-transformed into a host cell together with the expression vector containing hHGF gene. Such vectors may include pSV2neo (J. Mol. Appl. Genet., 1, 327 (1982)); pMBG (Nature, 294, 228 (1981)); pSV2gpt (Proc. Natl. Acad. Sci. USA, 78, 2072 (1981)); and pAd-D26-1 (J. Mol. Biol., 159, 601 (1982)). In this case, a transformant may be easily selected on the basis of the phenotype of the selective marker used.

In the above mentioned selection methods, those cells containing the desired hHGF protein gene may be repeatedly subjected to co-transformation using a different selective marker. This may preferably increase about 20 fold the amount of protein expressed.

Introduction of the expression vector into animal cells may be conducted by the calcium phosphate method (Virology, 52, 456 (1973)) or the electroporation method (J. Membr. Biol., 10, 279 (1972)). The calcium phosphate method is commonly used.

Animal cells thus transformed may be cultured in conventional manners by suspension or adhesion culture. MEM or RPMI1640 may be used as a culture medium and the culture may be carried out in the absence of presence of 5-10% serum and in the presence of an appropriate amount of insulin, dexamethasone or transferrin.

The animal cells producing the hHGF protein will secrete the produced hHGF protein into culture media. The hHGF protein can be purified and isolated from the supernatant of the culture. Specifically, the supernatant may be subjected to a combination of various chromatographic operations on S-Sepharose, heparin-Sepharose, hydroxyapatite, and/or sulfated cerulophan to purify and isolate hHGF protein.

According to the present invention, hHGF (pre-hHGF) having the amino acid sequence starting from Met as shown in Fig. 1 is first expressed in host cells. The hHGF (pre-hHGF) is then hydrolyzed between the 31st Gly and the 32nd Gln in the host cells; thus, the signal peptide of 31 amino acids is cleaved off. The N-terminal Gln is then deaminated to be converted into pyroglutamic acid. Thus, hHGF having pyroglutamic acid residue at its N-terminus is secreted.

In the hHGF of the present invention, the peptide chain from the N-terminal pyroglutamic acid to the 494th Arg constitutes the heavy (H) chain while the remaining peptide from the 495th Val to the last Ser the light (L) chain.

In accordance with the present invention, the hHGF protein having biological activities can be produced abundantly, stably and easily by introducing, into a host cell, an expression vector into which the hHGF gene of the present invention has been inserted. Such production of hHGF has not been attained prior to the present invention. Thus obtained recombinant hHGF, hHGF-like substances or hHGF-containing fused proteins may be used in the treatment of hepatic diseases as hepatic regeneration-enhancing, hepatic function-improving, hepatitis-curing, hepatic cirrhosis-suppressing agents.

EXAMPLES

The following examples will be given by way of illustration only. The present invention is not limited to these examples. It should be understood that those skilled in the art may make many modifications without departing from the spirit and scope of the present invention as defined in the attached claims.

Example 1

[1] Determination of partial amino acid sequence of hHGF and preparation of a probe

Purification of hHGF from plasma of patients with fulminant hepatitis was performed in accordance with the method described in *J. clin. Invest.*, 81, 414 (1988). Thus purified hHGF preparation was subjected to SDS-PAGE. A relatively broad single band was observed at molecular weights from 76,000 to 92,000 under non-reducing condition. SDS-PAGE under reducing condition, on the contrary, has revealed two bands; a relatively broad band at molecular weights of 56,000 to 65,000 and the other band at molecular weights of 32,000 to 35,000. A 50 µg portion of the purified hHGF preparation was dissolved in 100 µl of 50 mM Tris-HCl buffer (pH 9) containing 5M urea, and the resulting solution was mixed with an amount of *Achromobacter* Protease I equivalent to 1/200 of hHGF in molar ratio, followed by incubation at 37°C for 6 hours. The resulting peptide mixture was subjected to reduction and carboxymethylation in convention manners. Each peptide was then separated and isolated by means of reverse-phase high-pressure liquid chromatography using Bakerbond WP Octyl Column (J. T. Baker). Analyses of four peptides using a gas phase protein sequencer (Applied Biosystems; Model 470A) revealed their amino acid sequences as shown in the following Table 1.

Table 1:

Peptide No.	Amino acid sequences of peptides	
	Sequence	
1.	Phe Leu Pro Glu Arg Tyr Pro Asp Lys	
2.	Glu Phe Gly His Glu Phe Asp Leu Tyr Glu Asn Lys	
3.	Asp Tyr Glu Ala Trp Leu Gly Ile His Asp Val His Gly Arg Gly Asp XXX*Lys	
4.	Asn Met Glu Asp Leu His Arg His Ile Phe Trp Glu-Pro Asp Ala Ser Lys	
5.	Arg Arg Asn Thr Ile His Glu Phe Lys	
6.	Ile Asp Pro Ala Leu Lys	

*XXX indicates an amino acid not determined

Next, synthetic oligonucleotides were prepared based on the partial amino acid sequences, Asn-Met-Glu-Asp-Leu-His and His-Ile-Phe-Trp-Glu-Pro, of the peptide No. 4 shown in Table 1. That is, 64 oligonucleotides TH23 consisting of 17 bases (5'-T-G-T-C/A/G-A-A/G-A/G-T-C-T-C-T-C-C-A-T-A/G-T-T-3') and 24 oligonucleotides TH24 consisting of 17 bases (5'-G-G-T-C-T-C-C-C-A/A/G-A-A-A-G-T-A-T-A/G-T-G-

3') were prepared. The 5' end of each synthetic oligonucleotide was labeled with ^{32}P in a conventional manner using polynucleotide kinase in a reaction solution (50 mM Tris-HCl (pH 7.5), 10 mM magnesium chloride, 10 mM mercaptoethyl alcohol, 100 μM [γ - ^{32}P] ATP and substrate DNA). Unnecessary mononucleotides in these labeled samples were removed by conventional DEAE cellulose column chromatography.

[2] Screening of cDNA coding for a portion of hHGF

(1) Plaque hybridization

λ phage cDNA library (Clontech Laboratories, Inc.) originated from 34-week-old human placenta was screened according to the manufacturer's specifications. *E. coli* Y-1090 strain was infected with phages of 1,000,000 clones and cultured at 42°C overnight on an NZY soft agar medium using five Petri dishes (24.5 cm x 24.5 cm), each dish containing 200,000 clones (NZY medium; 1% NZ-Amine, 0.5% yeast extracts and 0.5% sodium chloride, adjusted to pH 7.5 and supplemented with 0.25% magnesium chloride, and NZY soft agar medium; NZY medium supplemented with agar powder to its final concentration of 0.7% and autoclaved).

Next, the resulting λ phage clones grown on the medium were transferred on a commercial nylon membrane (Gene Screening Plus, Du Pont Company) and subjected to plaque hybridization as follows. Phage particles grown on one dish were transferred on two nylon membranes and each membrane was put on a filter paper impregnated with 0.1 M sodium hydroxide and 1.5 M sodium chloride. After standing still for 2 minutes on the filter paper, moisture of the nylon membrane was removed by using another dry filter paper. The thus dried membrane was then placed on another filter paper impregnated with 2 x SSC - 0.2 M Tris-HCl (pH 7.4), stood still on the filter paper and then air-dried on another dry filter paper. These procedures were repeated again. The term "2 x SSC" means double concentration of SSCP solution and similar way of expressions are used hereinafter (10 x SSC; 1.2 M sodium chloride, 150 mM sodium citrate, 130 mM potassium dihydrogenphosphate and 1 mM EDTA and pH 7.2).

Thus treated nylon membrane was washed at 60°C for 15 minutes with 3 x SSC - 0.1% SDS (20 x SSC; 3 M sodium chloride and 0.3 M sodium citrate). The washing process was repeated again. Each of the thus washed nylon membrane was then incubated at 65°C for 3 hours in 5 ml of a pre-hybridization solution [3 x SSC, 0.1% SDS, 10 x Denhalt (50 x Denhalt solution; 1% BSA (bovine serum albumin), 1% polyvinyl pyrrolidone and 1% Ficoll 400), and 20 $\mu\text{g}/\text{ml}$ of salmon sperm DNA]. The foregoing nylon membranes were incubated for 36 hours in a hybridization solution containing the ^{32}P -labeled synthetic oligonucleotide probe prepared in [1] above [3 x SSC, 10 x Denhalt, 50 $\mu\text{g}/\text{ml}$ of salmon sperm DNA, 1 M sodium chloride, 1% SDS, 250 $\mu\text{g}/\text{ml}$ of salmon sperm DNA and 100,000 c.p.m./ml of ^{32}P -labeled probe DNA per each synthesized probe]. The incubation temperature was calculated by regarding A or T as 2°C and G or C as 4°C and totaling these values of each probe (42°C in the case of the TH23 probe and 46°C in the case of the TH24 probe). Thereafter, the thus incubated nylon membranes were removed from the hybridization solution, washed twice in 4 x SSC (30 minutes for each) at room temperature, washed twice in 4 x SSC (30 minutes for each) at the foregoing hybridization temperature, again washed twice in 2 x SSC (15 minutes for each) at room temperature, and then subjected to autoradiography.

A total of 6 autoradiographic signals which coincided with one another between a pair of the nylon membranes were found. In order to isolate clones corresponding to these signals, each of the plaques on the foregoing soft agar medium, which coincided with these positive signals, was removed using a glass tube. Phage particles in the thus removed plaque were extracted by incubating the plaque overnight in 1 ml of TMG buffer [50 mM Tris-HCl (pH 7.5), 100 mM sodium chloride, 10 mM magnesium chloride and 0.01% gelatin] in the presence of 50 μl of chloroform. *E. coli* Y-1090 cells were infected with the thus extracted phage particles, and an appropriate amount of the infected cells was cultured on the soft agar medium in a Petri dish (9 cm in diameter). The plaque hybridization was carried out as previously described. By repeating a series of these procedures, each clone corresponding to the positive autoradiographic signal was isolated.

As a result, a total of 6 independent clones were obtained. Of these, two clones, named λ hHGF21 and λ hHGF502, were subjected to the analysis of their cDNA base sequences.

(2) Subcloning of cDNA fragments and determination of base sequences

DNA fragments were extracted from the λ phage clones and subcloned into plasmid vectors pUC18 and pUC19 and single strand phage vectors M13mp18 and M13mp19 as follows.

Infection of $40 \mu\text{l}$ suspension of 2×10^8 cells of *E. coli* Y-1090 strain with 2×10^7 p.f.u. (plaque formation unit) of λ phage clone suspended in $200 \mu\text{l}$ of TMG solution was performed by incubating them at 37°C for 15 minutes in 200 ml of NZY medium in a 500 ml conical flask. Immediately after the incubation, 1 ml of 1 M calcium chloride solution was added and the culture was incubated overnight (about 14 hours).

To the culture was added 2 ml of chloroform. After standing still for about 10 minutes, 15.6 g of sodium chloride was added and dissolved. Centrifugation was carried out at $6,000 \text{ rpm}$ for 20 minutes at 4°C using a refrigerated centrifuge (model SCR 20BB; rotor, RPR 9-2; Hitachi Ltd.). A 20 g portion of polyethylene glycol 6,000 was added to the resulting supernatant fluid and dissolved thoroughly. After standing still for 1 hour in an ice bath, the resulting mixture was centrifuged at $6,000 \text{ rpm}$ for 20 minutes using Hitachi refrigerated centrifuge, model SCR 20BB, and the rotor, RPR 9-2. Resulting pellet was suspended in 6 ml of buffer A [0.5% NP40, 36 mM calcium chloride, 30 mM Tris-HCl (pH 7.5), 50 mM magnesium chloride, 125 mM potassium chloride, 0.5 mM EDTA, 0.25% deoxycholic acid and 0.6 mM mercaptoethanol]. The suspension was then mixed with $100 \mu\text{l}$ of 10 mg/ml Deoxyribonuclease I and $10 \mu\text{l}$ of 10 mg/ml Ribonuclease A and incubated at 30°C for 30 minutes in order to hydrolyze *E. coli*-originated nucleic acids. Thereafter, the reaction mixture was mixed with an equal volume of chloroform and stirred thoroughly followed by centrifugation $3,000 \text{ rpm}$ for 10 minutes (model LC-06; rotor, TS-7; Tomy Seiko Co., Ltd.) to obtain supernatant fluid.

On the other hand, a double-layer glycerol solution was prepared in a centrifugal tube for ultracentrifugation (rotor, RPS40T; Hitachi Ltd.) by firstly charging the tube with 1 ml of a 40% glycerol solution [0.5% NP40, 30 mM Tris-HCl (pH 7.5), 125 mM potassium chloride, 0.5 mM EDTA, 0.6 mM mercaptoethanol and 40% glycerol] and then by placing thereon 3 ml of a 10% glycerol solution [0.5% NP40, 30 mM Tris-HCl (pH 7.5), 125 mM potassium chloride, 0.5 mM EDTA, 0.6 mM mercaptoethanol and 10% glycerol]. On the double-layer solution was overlaid the nuclease-treated phage suspension. After centrifugation at $35,000 \text{ rpm}$ for 1 hour (model 70P72; rotor, RPS40T; Hitachi Ltd.), phage particles recovered as the pellet in the tube were suspended in 0.4 ml of 40 mM Tris-HCl (pH 7.5), 10 mM EDTA and 2% SDS and the suspension was incubated at 55°C for 1 hour in the presence of $4 \mu\text{l}$ of 10 mg/ml Proteinase K. Thereafter, the resulting solution was transferred into an Eppendorf tube, and the phage DNA was extracted with an equal volume of phenol/chloroform and recovered by ethanol precipitation. In this way, $200 \mu\text{g}$ of the phage DNA was obtained.

The phage DNA was digested with restriction enzyme *EcoRI* in a conventional manner and the digests were analyzed by agarose gel electrophoresis. Three *EcoRI* fragments of 0.2 kb , 0.85 kb and 0.72 kb in size were obtained from the clone, λ hHGF21. On the other hand, cDNA fragments were obtained by recovering the insert cDNA fragments from the agarose gel by conventional methods.

A 100 ng portion of each of these cDNA fragments and a 200 ng portion of each of plasmid vectors pUC18 and pUC19 and single strand phage vectors M13mp18 and M13mp19, which had been digested in advance with restriction enzyme *EcoRI* in conventional manners, were incubated in the presence of T4 DNA ligase in $10 \mu\text{l}$ of a reaction solution [66 mM Tris-HCl (pH 7.6), 6.6 mM magnesium chloride, 10 mM dithiothreitol, $66 \mu\text{M}$ ATP and substrate DNA]. Each of the ligated DNA samples was used to transform *E. coli* host selected in accordance with the used vector by conventional methods. As a result, subclones containing a partial base sequence of HGF gene in the *EcoRI* insert site were obtained.

Determination of base sequences of the cDNA subclones was performed in accordance with Sanger *et al.* dideoxy chain termination method. Primers which corresponded to commercially available M13 phage vectors were selected.

Deduction of amino acid sequence from the base sequence of the clone λ hHGF21 which had the longest cDNA revealed that the amino acid sequence of this clone contained some of the already determined partial amino acid sequence, which were different from the amino acid sequence used for the construction of probe, thus showing that this clone comprised a cDNA coding for at least a part of the hHGF.

Further, when cDNA base sequence of another clone λ hHGF502 which contained a different cDNA fragment that did not exist in the λ hHGF21, was analyzed in accordance with Sanger *et al.* method, it was found that the phage clone λ hHGF502 possessed a common 0.8 kb base sequence of the phage clone λ hHGF21, i.e., the sequence which ranged from a base around the restriction enzyme *NcoI* site to a base around the third *EcoRI* cleavage site from $5'$ upstream as shown in Fig. 2, as well as a 0.7 kb base sequence at $3'$ side of the common sequence, which was not found in λ hHGF21. It was also found that the base sequence of λ hHGF502, which was not included in the base sequence of λ hHGF21, contained a partial base sequence which corresponded to one of the already determined partial amino acid sequence of

hHGF. It was further found that the whole amino acid sequence of hHGF was covered up by combining the base sequences of these two clones in such a way that the common parts of their base sequences were overlapped.

EXAMPLE 2:

(I) Preparation of hHGF Expression Plasmid

Fig. 3 shows the scheme used for the preparation of an hHGF expressing plasmid.

According to the conventional method described in "Molecular Cloning", Cold Spring Harbor Laboratory, p.93 (1982), plasmid pUCHGF-1 DNA was prepared, which comprised a Bam HI-Kpn I fragment containing hHGF cDNA (Biochem. Biophys. Res. Commun., 163(2), 967-973 (1989)). The Bam HI-Kpn I fragment of about 2.3 kb in size extended from the Bam HI site at 27 base upstream of the initiation codon ATG to the Kpn I site at 8 base upstream of the stop codon TAG.

The plasmid DNA (10 ug) was digested with Kpn I restriction enzyme in a conventional manner. The resulting DNA fragment was extracted with phenol/chloroform, purified by ethanol precipitation, and dissolved in 10 μ l water.

Into the Kpn I cleavage site of the DNA fragment, a synthetic linker of 32 bases was introduced according to Maniatis et al. method described in "Molecular Cloning", Cold Spring Harbor Laboratory, 396-397 (1982)). The linker had Kpn I site at both ends thereof and contained therein a stop codon TGA and a Bam HI cleavage site, as shown in Fig. 3.

The thus modified plasmid was used to transform *E. coli* in a conventional manner. From the resulting transformants, plasmid DNA was prepared in accordance with the conventional method described in "Molecular Cloning", Cold Spring Harbor Laboratory, p.93 (1982).

The plasmid DNA (10 ug) was digested with Bam HI restriction enzyme in a conventional manner. The resulting reaction mixture was subjected to 1.0% agarose gel electrophoresis to isolate the hHGF DNA fragments containing ATG initiation and TGA stop codons from concomitant undesirable DNA fragments.

From the agarose gel, Bam HI-Bam HI DNA fragment of about 2.3 kb coding for hHGF was prepared according to Maniatis et al. method described in "Molecular Cloning", Cold Spring Harbor Laboratory, p.164 (1982). The DNA fragment was treated with T4 DNA polymerase in a conventional manner to form blunt ends at both termini thereof. The blunt-ended DNA fragment was extracted with phenol/chloroform, purified by ethanol precipitation, and dissolved in 10 μ l water.

On the other hand, 0.05 ug of expression vector pKCR as described in Proc. Natl. Acad. Sci., 78, 1527 (1981) was digested with Sma I restriction enzyme to form blunt ends in a conventional manner, extracted with phenol/chloroform, and purified by ethanol precipitation. The vector DNA was dissolved in 400 μ l of 50 mM Tris-HCl (pH 8), 1 mM magnesium chloride, and dephosphorylated with 1 unit of bacterial alkaline phosphatase (TOYOBO, BAP-101) at 65°C for 30 minutes. The DNA fragment was then extracted with phenol/chloroform, purified by ethanol precipitation, and dissolved in 10 μ l water.

The vector pKCR DNA fragment (0.01 ug) prepared above was ligated to the blunt-ended Bam HI hHGF cDNA fragment (0.1 ug) in the presence of T4 DNA ligase (TOYOBO, LGA-101) in 20 μ l of 66 mM Tris-HCl (pH 7.6), 6.6 mM magnesium chloride, 10 mM dithiothreitol, 66 μ M ATP at 14°C for 12 hours.

The resulting reaction mixture (10 μ l) was used to transform *E. coli* HB 101 (Takara Shuzo) according to the specification. The transformants were cultured on a medium containing 50 ug/ml ampicillin. Several tens of ampicillin resistant strains were obtained.

Plasmids from these strains were analyzed in accordance with Maniatis et al. method described in "Molecular Cloning", Cold Spring Harbor Laboratory, 86-96 (1982). Thus, plasmid pKCRHGF-2 was obtained, in which two hHGF genes had been incorporated in tandem into the Sma I cleavage site present between the promoter and polyadenylation signal in the expression vector pKCR.

The structure of the plasmid pKCRHGF-2 is shown in Fig. 4.

(II) Preparation of Cell Strain Expressing Stably After Passages

According to Maniatis et al. method described in "Molecular Cloning", Cold Spring Harbor Laboratory, 86-96 (1982), plasmid pKCRHGF-2 prepared in (I) above, in which two hHGF cDNA fragments had been inserted into the Bam HI cleavage site of pKCR (Proc. Natl. Acad. Sci., 78(2), 1527 (1981)), was recovered

from the recombinant *E. coli* and purified to obtain a large amount of hGF expression plasmid DNA.

On the other hand, plasmid pSV2neo DNA (J. Appl. Genet., 1, 327 (1982)) and plasmid pAd-D26-1 DNA (J. Molec. Biol., 159, 601 (1982)), each coding for a marker for selecting transformants, were recovered from recombinant *E. coli* strains containing the respective plasmid and purified, in accordance with the
5 aforementioned Maniatis et al. method.

Three plasmids thus prepared were used to co-transform CHO cells in accordance with Ausubel et al. method described in "Current Protocols in Molecular Biology", Greene Publishing Associates and Wiley-Interscience, Chapters 9.1.1 to 9.1.4, (1987).

First, CHO cells were cultured to semi-confluent in ERDF medium (Kyokuto Seiyaku, Japan) containing
10 10% FCS (fetal calf serum) in a Petri dish of 9 cm in diameter. The medium was removed from the dish and there was added dropwise a DNA solution, which had previously been prepared in the following manner:

In an Eppendorf centrifugal tube, 300 μ l of 2xHEBS solution (1.6% sodium chloride, 0.074% potassium chloride, 0.05% disodium hydrogenphosphate dodecahydrate, 0.2% dextrose, 1% HEPES (pH 7.05)), 10 μ g
15 of the expression plasmid DNA, 1 μ g of pSV2neo plasmid DNA, and 1 μ g of pAd-D26-1 plasmid DNA were added per each Petri dish of 9 cm in diameter and sterilized water added to 570 μ l in total volume. To the DNA solution, 30 μ l of 2.5 M calcium chloride solution was added dropwise while blending vigorously on a Vortex mixer for a few seconds. The resulting mixture was allowed to stand at room temperature for 30 minutes while mixing by Vortex mixer each 10 minutes.

This DNA solution was added to the semi-confluent cells in Petri dish and the cells were allowed to stand at room temperature for 30 minutes. Then, 9 ml of ERDF medium containing 10% FCS was added to the dish followed by culturing in the presence of 5% CO₂ at 37°C for 4 to 5 hours.

The media was removed from the dish and the cells were washed with 5 ml of 1xTBS++ solution (25 mM Tris-HCl, pH 7.5, 140 mM sodium chloride, 5 mM potassium chloride, 0.6 mM disodium hydrogen-
25 phosphate, 0.08 mM calcium chloride, 0.08 mM magnesium chloride). After removing the 1xTBS++ solution, 5 ml of 1xTBS++ solution containing 20% glycerol was added to the cells. The cells were allowed to stand at room temperature for 1 to 2 minutes. After removing the supernatant, the cells were again washed with 5 ml of 1xTBS++ solution. Thereafter, 10 ml of ERDF medium containing 10% FCS was added to the Petri dish followed by culturing in the presence of 5% CO₂ at 37°C.

After 48 hours culture, the medium was removed from the dish and the cells were washed with 5 ml of
30 1xTBS++ solution. Then, 2 ml of trypsin-EDTA solution (Sigma) was added to the cells and the mixture was allowed to stand at room temperature for 30 seconds. The trypsin-EDTA solution was then removed from the dish. After 5 minutes, 10 ml of ERDF medium containing 10% FCS was added to the dish to strip off the cells. The cultured cells of one Petri dish of 9 cm in diameter were divided into 10 portions and each
35 portion was placed in a Petri dish of 9 cm in diameter. G418 sulphate (GENETICIN, GIBCO) was added to each dish to 200 μ g/ml and the cells were further cultured.

Ten days later, surviving G418-resistant cells were isolated and distributed in wells of a 24-well culture plate, each well of 3.1 cm² containing 1 ml of ERDF medium + 10% FCS, followed by further culturing for approximately 7 days.

The medium was replaced by FCS-free ERDF medium and the culture was continued for further 72
40 hours. Then, 2 ml of the medium was recovered from each well and concentrated to 50 μ l by Centricon (Millipore). About 15 μ l of the sample was subjected to electrophoresis on SDS-polyacrylamide gel.

These samples were analyzed by conventional Western blotting method to confirm the expression of hHGF protein. The presence of the biological activity was also confirmed by measuring hHGF activity

45 according to Gohda et al. method described in Exp. Cell Res., 166, 139-150 (1986).
Further, the cells obtained were isolated from each well and quantitative measurement of hHGF protein was performed by enzyme immunoassay. The amount of hHGF expressed was determined in B-1, B-27 and B-102 cells, which produced a significantly large amount of protein.

50 EXAMPLE 3: Preparation of Cell Strain Expressing Stably After Passages by Double Transformation

Plasmid DNAs of the expression vector pKCRHGF-2 prepared in (I) above and pMBG coding for mycophenolic acid resistance as a marker for selecting transformants (Nature, 294, 228 (1981)) were
55 recovered from recombinant *E. coli* strains containing the respective plasmid and purified, in accordance with the aforementioned Maniatis et al. method.

The resulting two plasmids were used to again co-transform each of those cells B-1, B-27 and B-102 which was isolated in Example 2 (II) and capable of expressing a large amount of hHGF stably after

passages, in accordance with Ausubel et al. method described in "Current Protocols in Molecular Biology", Greene Publishing Associates and Wiley-Interscience, Chapters 9.1.1 to 9.1.4. (1987).

First, the hHGF-expressing cells were cultured to semi-confluent in ERDF medium containing 10% FCS in a Petri dish of 9 cm in diameter. The medium was then removed from the dish and there was added dropwise the DNA solution, which had previously been prepared in the same manner as in Example 2 (II) except that 10 µg of pKORHGF-2 plasmid DNA and 1 µg of pMBG plasmid DNA were used.

This DNA solution was added to the semi-confluent cells in Petri dish and the cells were allowed to stand at room temperature for 30 minutes. Then, 9 ml of ERDF medium containing 10% FCS was added to the dish followed by culturing in the presence of 5% CO₂ at 37°C for 4 to 5 hours.

The medium was removed from the dish and the cells were washed with 5 ml of 1xTBS++ solution. After removing the 1xTBS++ solution, 5 ml of 1xTBS++ solution containing 20% glycerol was added to the cells. The cells were allowed to stand at room temperature for 1 to 2 minutes. After removing the supernatant, the cells were again washed with 5 ml of 1xTBS++ solution. Thereafter, 10 ml of ERDF medium containing 10% FCS was added to the Petri dish followed by culturing in the presence of 5% CO₂ at 37°C.

After 48 hours culture, the medium was removed from the dish and the cells were washed with 5 ml of 1xTBS++ solution. Then, 2 ml of trypsin-EDTA solution (Sigma) was added to the cells and the mixture was allowed to stand at room temperature for 30 seconds. The trypsin-EDTA solution was then removed from the dish. After 5 minutes, 10 ml of alpha-MEM(-) medium containing 10% FCS was added to the dish to strip off the cells. The cultured cells of one Petri dish of 9 cm in diameter were divided into 10 portions and each portion was placed in a Petri dish of 9 cm in diameter. Mycophenolic acid (Sigma) and xanthine (Sigma) were added to each dish to 1 µg/ml and 250 µg/ml, respectively, and the cells were further cultured.

Ten days later, surviving mycophenolic acid-resistant cells were isolated and distributed in wells of a 24-well culture plate, each well of 3.1 cm² containing 1 ml of ERDF medium + 10% FCS, followed by further culturing for approximately 7 days.

The medium was replaced by FCS-free ERDF medium and the culture was continued for further 72 hours. Then, 2 ml of the medium was recovered from each well and concentrated to 50 µl by Centricon (Millipore). About 15 µl of the sample was subjected to electrophoresis on SDS-polyacrylamide gel.

These samples were analyzed by conventional Western blotting method to confirm the expression of hHGF protein. The presence of the biological activity was also confirmed by measuring hHGF activity according to Gohda et al. method described in Exp. Cell Res., 166, 139-150 (1986). The results are shown in Fig. 5.

Further, some of the obtained cells were isolated and the amount of hHGF protein expressed was measured by enzyme immunoassay. There was obtained doubly transformed BD-24 cell exhibiting the amount of hHGF expressed, which was 20-fold that of single transformant B-102 cell.

EXAMPLE 4:

The hHGF producing cell BD-24 prepared in Example 3 was cultured in ERDF medium containing 10% FCS. The supernatant (500 ml) was adsorbed on a column filled with 10 ml S-Sepharose Fast Flow™ (Pharmacia). The proteins were eluted using 10 mM sodium phosphate-containing buffer (pH 7.5) with increasing concentrations of sodium chloride therein. Recombinant hHGF protein was eluted with approximately 0.7 M sodium chloride.

This hHGF fraction was analyzed by SDS-polyacrylamide gel electrophoresis yielding a broad band with molecular weights of about 76,000 to 92,000 under non-reducing condition and on the other hand a broad band of about 60,000 to 65,000 and a weak band of about 56,000 under reducing condition, which corresponded to H chain of hHGF protein, further giving two bands with molecular weights of about 32,000 to 35,000, which corresponded to L chain of hHGF protein. These band multiplicity and broadness may arise from heterogeneity of glycosylated sugar chains on hHGF protein.

The buffer of the purified hHGF protein solution was substituted by 0.1 M aqueous ammonium bicarbonate solution. This solution was mixed with 1/50 volume of *Staphylococcus aureus* V8 protease (Miles Laboratory) and incubated at 37°C overnight to yield a peptide mixture. This mixture was subjected to reverse phase high pressure liquid chromatography using C8 column (Bakerbond, 4.6 x 250 mm) while increasing acetonitrile concentration from 0% to 60% at a rate of 1% per minute.

Approximately 10 peptide peaks eluted were subjected to amino acid analysis to reveal that the peak eluted at about 18 minutes has the amino acid composition as shown in Table below.

Table:

Amino Acid Composition	
Aspartic acid/Asparagine	1.22
Threonine	0.63
Glutamic acid/Glutamine	2.03
Isoleucine	0.99
Lysine	0.92
Histidine	0.65
Arginine	3.11

5
15 The composition shown in Table substantially coincides with the theoretical composition of a peptide extending from the 32nd glutamine to the 41st glutamic acid, as calculated from the first methionine, in the amino acid sequence (Fig. 1) deduced from the base sequence of cDNA coding for hHGF (Fig. 2).

This peptide was analyzed by fast atom bombardment massspectroscopy (NIHON DENSHI, Japan, model HX-100). A peak was found at mass 1321 indicating that the peptide had a molecular weight of 1320. Since the theoretical molecular weight of the peptide extending from the 32nd glutamine to the 41st glutamic acid is 1337 of the amino acid sequence shown in Fig. 1, it can be concluded that the amino
20 terminal glutamine of this peptide is converted by deamination into pyroglutamic acid.

Thus, the N terminal amino acid of the secreted hHGF protein is found to be pyroglutamic acid derived from the 32nd amino acid glutamine in the amino acid sequence shown in Fig. 1.

25

Claims

1. Hepatic parenchymal cell growth factor represented by the following amino acid sequence:

30

35

40

45

50

55

	Met	Trp	Val	Thr	Lys	Leu	Leu	Pro	Ala	Leu
	Leu	Leu	Gln	His	Val	Leu	Leu	His	Leu	Leu
5	Leu	Leu	Pro	Ile	Ala	Ile	Pro	Tyr	Ala	Glu
	Gly	Gln	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His
10	Glu	Phe	Lys	Lys	Ser	Ala	Lys	Thr	Thr	Leu
	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys
15	Thr	Lys	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys
	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu
20	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp
	Lys	Ala	Arg	Lys	Gln	Cys	Leu	Trp	Phe	Pro
25	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys
	Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu
30	Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	Ile	Ile
	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val
35	Ser	Ile	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln
	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His
40	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys
	Asp	Leu	Gln	Glu	Asn	Tyr	Cys	Arg	Asn	Pro
45	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe
	Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val
50	Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	Val	Glu
55										

	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg
	Gly	Leu	Met	Asp	His	Thr	Glu	Ser	Gly	Lys
5	He	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro
	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr
10	Pro	Asp	Lys	Gly	Phe	Asp	Asp	Asn	Tyr	Cys
	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp
15	Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp
	Glu	Tyr	Cys	Ala	Tyr	Lys	Thr	Cys	Ala	Asp
	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu
20	Glu	Thr	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly
	Ile	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile
25	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp
	Ser	Gln	Tyr	Pro	His	Glu	His	Asp	Met	Thr
30	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg
	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser
	Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro
35	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile
	Pro	Asn	Cys	Asp	Met	Ser	His	Gly	Gln	Asp
40	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met
	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu
45	Thr	Cys	Ser	Met	Trp	Asp	Lys	Asn	Met	Glu
	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro
	Asp	Ala	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys
50	Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	Gly	Pro
	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro
55	Trp	Asp	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu

	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu
5	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr
	Lys	Gln	Leu	Arg	Val	Val	Asn	Gly	Ile	Pro
	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser
10	Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly
	Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	Val	Leu
15	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp
	Leu	Lys	Asp	Tyr	Glu	Ala	Trp	Leu	Gly	Ile
	His	Asp	Val	His	Gly	Arg	Gly	Asp	Glu	Lys
20	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu
	Val	Tyr	Gly	Pro	Glu	Gly	Ser	Asp	Leu	Val
25	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu
	Asp	Asp	Phe	Val	Ser	Thr	Ile	Asp	Leu	Pro
30	Asn	Tyr	Gly	Cys	Thr	Ile	Pro	Glu	Lys	Thr
	Ser	Cys	Ser	Val	Tyr	Gly	Trp	Gly	Tyr	Thr
	Gly	Leu	Ile	Asn	Tyr	Asp	Gly	Leu	Leu	Arg
35	Val	Ala	His	Leu	Tyr	Ile	Met	Gly	Asn	Glu
	Lys	Cys	Ser	Gln	His	His	Arg	Gly	Lys	Val
40	Thr	Leu	Asn	Glu	Ser	Glu	Ile	Cys	Ala	Gly
	Ala	Glu	Lys	Ile	Gly	Ser	Gly	Pro	Cys	Glu
45	Gly	Asp	Tyr	Gly	Gly	Pro	Leu	Val	Cys	Glu
	Gln	His	Lys	Met	Arg	Met	Val	Leu	Gly	Val
	Ile	Val	Pro	Gly	Arg	Gly	Cys	Ala	Ile	Pro
50	Asn	Arg	Pro	Gly	Ile	Phe	Val	Arg	Val	Ala
	Tyr	Tyr	Ala	Lys	Trp	Ile	His	Lys	Ile	Ile
55	Leu	Thr	Tyr	Lys	Val	Pro	Gln	Ser	*	

2. Hepatic parenchymal cell growth factor represented by the following amino acid sequence extending from the 30th glutamic acid to the last serine in the sequence defined in claim 1:

5										Glu
	Gly	Gln	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His
10	Glu	Phe	Lys	Lys	Ser	Ala	Lys	Thr	Thr	Leu
	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys
15	Thr	Lys	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys
	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu
20	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp
	Lys	Ala	Arg	Lys	Gln	Cys	Leu	Trp	Phe	Pro
	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys
25	Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu
	Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	Ile	Ile
30	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val
	Ser	Ile	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln
35	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His
	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys
40	Asp	Leu	Gln	Glu	Asn	Tyr	Cys	Arg	Asn	Pro
	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe
45	Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val
	Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	Val	Glu
	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg
50	Gly	Leu	Met	Asp	His	Thr	Glu	Ser	Gly	Lys
	Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro

55

	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr
	Pro	Asp	Lys	Gly	Phe	Asp	Asp	Asn	Tyr	Cys
5	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp
	Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp
10	Glu	Tyr	Cys	Ala	Tyr	Lys	Thr	Cys	Ala	Asp
	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu
15	Glu	Thr	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly
	Ile	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile
20	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp
	Ser	Gln	Tyr	Pro	His	Glu	His	Asp	Met	Thr
25	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg
	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser
30	Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro
	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile
35	Pro	Asn	Cys	Asp	Met	Ser	His	Gly	Gln	Asp
	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met
40	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu
	Thr	Cys	Ser	Met	Trp	Asp	Lys	Asn	Met	Glu
45	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro
	Asp	Ala	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys
50	Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	Gly	Pro
	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro
55	Trp	Asp	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu
	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu
	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr

	Lys	Gln	Leu	Arg	Val	Val	Asn	Gly	Ile	Pro
5	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser
	Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly
10	Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	Val	Leu
	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp
15	Leu	Lys	Asp	Tyr	Glu	Ala	Trp	Leu	Gly	Ile
	His	Asp	Val	His	Gly	Arg	Gly	Asp	Glu	Lys
20	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu
	Val	Tyr	Gly	Pro	Glu	Gly	Ser	Asp	Leu	Val
25	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu
	Asp	Asp	Phe	Val	Ser	Thr	Ile	Asp	Leu	Pro
30	Asn	Tyr	Gly	Cys	Thr	Ile	Pro	Glu	Lys	Thr
	Ser	Cys	Ser	Val	Tyr	Gly	Trp	Gly	Tyr	Thr
35	Gly	Leu	Ile	Asn	Tyr	Asp	Gly	Leu	Leu	Arg
	Val	Ala	His	Leu	Tyr	Ile	Met	Gly	Asn	Glu
40	Lys	Cys	Ser	Gln	His	His	Arg	Gly	Lys	Val
	Thr	Leu	Asn	Glu	Ser	Glu	Ile	Cys	Ala	Gly
45	Ala	Glu	Lys	Ile	Gly	Ser	Gly	Pro	Cys	Glu
	Gly	Asp	Tyr	Gly	Gly	Pro	Leu	Val	Cys	Glu
50	Gln	His	Lys	Met	Arg	Met	Val	Leu	Gly	Val
	Ile	Val	Pro	Gly	Arg	Gly	Cys	Ala	Ile	Pro
55	Asn	Arg	Pro	Gly	Ile	Phe	Val	Arg	Val	Ala
	Tyr	Tyr	Ala	Lys	Trp	Ile	His	Lys	Ile	Ile
	Leu	Thr	Tyr	Lys	Val	Pro	Gln	Ser	*	

3. Hepatic parenchymal cell growth factor represented by the following amino acid sequence extending from the 32nd glutamine to the last serine in the sequence defined in claim 1:

5		Gln	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His
	Glu	Phe	Lys	Lys	Ser	Ala	Lys	Thr	Thr	Leu
10	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys
	Thr	Lys	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys
	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu
15	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp
	Lys	Ala	Arg	Lys	Gln	Cys	Leu	Trp	Phe	Pro
20	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys
	Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu
25	Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	Ile	Ile
	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val
30	Ser	Ile	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln
	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His
	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys
35	Asp	Leu	Gln	Glu	Asn	Tyr	Cys	Arg	Asn	Pro
	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe
40	Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val
	Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	Val	Glu
45	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg
	Gly	Leu	Met	Asp	His	Thr	Glu	Ser	Gly	Lys
50	Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro

	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr
	Pro	Asp	Lys	Gly	Phe	Asp	Asp	Asn	Tyr	Cys
5	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp
	Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp
10	Glu	Tyr	Cys	Ala	Tyr	Lys	Thr	Cys	Ala	Asp
	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu
15	Glu	Thr	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly
	Ile	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile
	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp
20	Ser	Gln	Tyr	Pro	His	Glu	His	Asp	Met	Thr
	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg
25	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser
	Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro
	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile
30	Pro	Asn	Cys	Asp	Met	Ser	His	Gly	Gln	Asp
	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met
35	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu
	Thr	Cys	Ser	Met	Trp	Asp	Lys	Asn	Met	Glu
40	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro
	Asp	Ala	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys
45	Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	Gly	Pro
	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro
	Trp	Asp	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu
50	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu
	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr
55	Lys	Gln	Leu	Arg	Val	Val	Asn	Gly	Ile	Pro

	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser
5	Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly
	Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	Val	Leu
10	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp
	Leu	Lys	Asp	Tyr	Glu	Ala	Trp	Leu	Gly	Ile
15	His	Asp	Val	His	Gly	Arg	Gly	Asp	Glu	Lys
	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu
20	Val	Tyr	Gly	Pro	Glu	Gly	Ser	Asp	Leu	Val
	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu
25	Asp	Asp	Phe	Val	Ser	Thr	Ile	Asp	Leu	Pro
	Asn	Tyr	Gly	Cys	Thr	Ile	Pro	Glu	Lys	Thr
30	Ser	Cys	Ser	Val	Tyr	Gly	Trp	Gly	Tyr	Thr
	Gly	Leu	Ile	Asn	Tyr	Asp	Gly	Leu	Leu	Arg
35	Val	Ala	His	Leu	Tyr	Ile	Met	Gly	Asn	Glu
	Lys	Cys	Ser	Gln	His	His	Arg	Gly	Lys	Val
40	Thr	Leu	Asn	Glu	Ser	Glu	Ile	Cys	Ala	Gly
	Ala	Glu	Lys	Ile	Gly	Ser	Gly	Pro	Cys	Glu
45	Gly	Asp	Tyr	Gly	Gly	Pro	Leu	Val	Cys	Glu
	Gln	His	Lys	Met	Arg	Met	Val	Leu	Gly	Val
50	Ile	Val	Pro	Gly	Arg	Gly	Cys	Ala	Ile	Pro
	Asn	Arg	Pro	Gly	Ile	Phe	Val	Arg	Val	Ala
55	Tyr	Tyr	Ala	Lys	Trp	Ile	His	Lys	Ile	Ile
	Leu	Thr	Tyr	Lys	Val	Pro	Gln	Ser	*	

4. Hepatic parenchymal cell growth factor represented by the following amino acid sequence, wherein X denotes pyroglutamic acid:

		X	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His
	Glu	Phe	Lys	Lys	Ser	Ala	Lys	Thr	Thr	Leu
5	Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys
	Thr	Lys	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys
10	Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu
	Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp
15	Lys	Ala	Arg	Lys	Gln	Cys	Leu	Trp	Phe	Pro
	Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys
20	Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu
	Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	Ile	Ile
25	Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val
	Ser	Ile	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln
30	Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His
	Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys
35	Asp	Leu	Gln	Glu	Asn	Tyr	Cys	Arg	Asn	Pro
	Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe
40	Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val
	Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	Val	Glu
45	Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg
	Gly	Leu	Met	Asp	His	Thr	Glu	Ser	Gly	Lys
50	Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro
	His	Arg	His	Lys	Phe	Leu	Pro	Glu	Arg	Tyr

	Pro	Asp	Lys	Gly	Phe	Asp	Asp	Asn	Tyr	Cys
5	Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp
	Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp
10	Glu	Tyr	Cys	Ala	Tyr	Lys	Thr	Cys	Ala	Asp
	Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu
15	Glu	Thr	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly
	Ile	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile
20	Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp
	Ser	Gln	Tyr	Pro	His	Glu	His	Asp	Met	Thr
25	Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg
	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser
30	Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro
	Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile
35	Pro	Asn	Cys	Asp	Met	Ser	His	Gly	Gln	Asp
	Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met
40	Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu
	Thr	Cys	Ser	Met	Trp	Asp	Lys	Asn	Met	Glu
45	Asp	Leu	His	Arg	His	Ile	Phe	Trp	Glu	Pro
	Asp	Ala	Ser	Lys	Leu	Asn	Glu	Asn	Tyr	Cys
50	Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	Gly	Pro
	Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro
55	Trp	Asp	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu
	Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu
	Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr

	Lys	Gln	Leu	Arg	Val	Val	Asn	Gly	Ile	Pro
5	Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser
	Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly
10	Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	Val	Leu
	Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp
15	Leu	Lys	Asp	Tyr	Glu	Ala	Trp	Leu	Gly	Ile
	His	Asp	Val	His	Gly	Arg	Gly	Asp	Glu	Lys
20	Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu
	Val	Tyr	Gly	Pro	Glu	Gly	Ser	Asp	Leu	Val
25	Leu	Met	Lys	Leu	Ala	Arg	Pro	Ala	Val	Leu
	Asp	Asp	Phe	Val	Ser	Thr	Ile	Asp	Leu	Pro
30	Asn	Tyr	Gly	Cys	Thr	Ile	Pro	Glu	Lys	Thr
	Ser	Cys	Ser	Val	Tyr	Gly	Trp	Gly	Tyr	Thr
35	Gly	Leu	Ile	Asn	Tyr	Asp	Gly	Leu	Leu	Arg
	Val	Ala	His	Leu	Tyr	Ile	Met	Gly	Asn	Glu
40	Lys	Cys	Ser	Gln	His	His	Arg	Gly	Lys	Val
	Thr	Leu	Asn	Glu	Ser	Glu	Ile	Cys	Ala	Gly
45	Ala	Glu	Lys	Ile	Gly	Ser	Gly	Pro	Cys	Glu
	Gly	Asp	Tyr	Gly	Gly	Pro	Leu	Val	Cys	Glu
50	Gln	His	Lys	Met	Arg	Met	Val	Leu	Gly	Val
	Ile	Val	Pro	Gly	Arg	Gly	Cys	Ala	Ile	Pro
55	Asn	Arg	Pro	Gly	Ile	Phe	Val	Arg	Val	Ala
	Tyr	Tyr	Ala	Lys	Trp	Ile	His	Lys	Ile	Ile
	Leu	Thr	Tyr	Lys	Val	Pro	Gln	Ser	*	

5. A gene coding for hepatic parenchymal cell growth factor represented by the amino acid sequence defined in claim 1.
6. A gene coding for hepatic parenchymal cell growth factor represented by the amino acid sequence defined in claim 2.
7. A gene coding for hepatic parenchymal cell growth factor represented by the amino acid sequence defined in claim 3.
8. The gene coding for hepatic parenchymal cell growth factor, which is represented by the following base sequence:

10

15

20

25

30

35

40

45

50

55

ATG TGG GTG ACC AAA CTC CTG CCA GCG CTG
CTG CTG CAG CAT GTC CTC CTG CAT CTC CTC
5 CTG CTC CCG ATC GCG ATC CCG TAT GCA GAG
GGA CAA AGG AAA AGA AGA AAT ACA ATT CAT
10 GAA TTC AAA AAA TCA GCA AAG ACT ACC CTA
ATC AAA ATA GAT CCA GCA CTG AAG ATA AAA
15 ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT
GCT AAT AGA TGT ACT AGG AAT AAA GGA CTT
20 CCA TTC ACT TGC AAG GCT TTT GTT TTT GAT
AAA GCA AGA AAA CAA TGC CTC TGG TTC CCC
TTC AAT AGC ATG TCA AGT GGA GTG AAA AAA
25 GAA TTT GGC CAT GAA TTT GAC CTC TAT GAA
AAC AAA GAC TAC ATT AGA AAC TGC ATC ATT
30 GGT AAA GGA CGC ACG TAC AAG GGA ACA GTA
TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG
35 CCC TGG AGT TCC ATG ATA CCA CAC GAA CAC
AGC TTT TTG CCT TCG AGC TAT CGG GGT AAA
40 GAC CTA CAG GAA AAC TAC TGT CGA AAT CCT
CGA GGG GAA GAA GGG GGA CCC TGG TGT TTC
45 ACA AGC AAT CCA GAG GTA CGC TAC GAA GTC
TGT GAC ATT CCT CAG TGT TCA GAA GTT GAA
50 TGC ATG ACC TGC AAT GGG GAG AGT TAT CGA

GGT CTC ATG GAT CAT ACA GAA TCA GGC AAG
ATT TGT CAG GGC TGG GAT CAT CAG ACA CCA
5 CAC GGG CAC AAA TTC TTG CCT GAA AGA TAT
CCC GAC AAG GGC TTT GAT GAT AAT TAT TGC
10 CGC AAT CCC GAT GGC CAG CCG AGG CCA TGG
TGC TAT ACT CTT GAC CCT CAC ACC CGC TGG
15 GAG TAC TGT GCA ATT AAA ACA TGC GGT GAC
AAT ACT ATG AAT GAC ACT GAT GTT CCT TTG
GAA ACA ACT GAA TGC ATC CAA GGT CAA GGA
20 GAA GGC TAC AGG GGC ACT GTC AAT ACC ATT
TGG AAT GGA ATT CCA TGT CAG CGT TGG GAT
25 TCT CAG TAT CCT CAC GAG CAT GAC ATG ACT
CCT GAA AAT TTC AAG TGC AAG GAC CTA CGA
30 GAA AAT TAC TGC CGA AAT CCA GAT GGG TCT
GAA TCA CCC TGG TGT TTT ACC ACT GAT CCA
AAC ATC CGA GTT GGC TAC TGC TCC CAA ATT
35 CCA AAC TGT GAT ATG TCA CAT GGA CAA GAT
TGT TAT CGT GGG AAT GGC AAA AAT TAT ATG
40 GGC AAC TTA TCC CAA ACA AGA TCT GGA CTA
ACA TGT TCA ATG TGG GAC AAG AAC ATG GAA
45 GAC TTA CAT CGT CAT ATC TTC TGG GAA CCA
GAT GCA AGT AAG CTG AAT GAG AAT TAC TGC
CGA AAT CCA GAT GAT GAT GCT CAT GGA CCC
50 TGG TGC TAC ACG GGA AAT CCA CTC ATT CCT
TGG GAT TAT TGC CCT ATT TCT CGT TGT GAA
55 GGT GAT ACC ACA CCT ACA ATA GTC AAT TTA

5
10
15
20
25
30
35
40
45
50
55

GAC CAT CCC GTA ATA TCT TGT GCG AAA ACG
AAA CAA TTG CGA GTT GTA AAT GCG ATT CCA
ACA CGA ACA AAC ATA GGA TGG ATG GTT AGT
TTG AGA TAC AGA AAT AAA CAT ATC TGC GGA
GGA TCA TTG ATA AAG GAG AGT TGG GTT CTT
ACT GCA CGA CAG TGT TTC CCT TCT CGA GAC
TTG AAA GAT TAT GAA GCT TGG CTT GGA ATT
CAT GAT GTC CAC GGA AGA GGA GAT GAG AAA
TGC AAA CAG GTT CTC AAT GTT TCC CAG CTG
GTA TAT GGC CCT GAA GGA TCA GAT CTG GTT
TTA ATG AAG CTT GCC AGG CCT GCT GTC CTG
GAT GAT TTT GTT AGT ACG ATT GAT TTA CCT
AAT TAT GGA TGC ACA ATT CCT GAA AAG ACC
AGT AGC AGT GTT TAT GGC TGG GGC TAC ACT
GGA TTG ATC AAC TAT GAT GGC CTA TTA CGA
GTG GCA CAT CTC TAT ATA ATG GGA AAT GAG
AAA TGC AGC CAG CAT CAT CGA GGG AAG GTG
ACT CTG AAT GAG TCT GAA ATA TGT GCT GGG
GCT GAA AAG ATT GGA TCA GGA CCA TGT GAG
GGG GAT TAT GGT GGC CCA CTT GTT TGT GAG
CAA CAT AAA ATG AGA ATG GTT CTT GGT GTC
ATT GTT CCT GGT CGT GGA TGT GCC ATT CCA
AAT CGT CCT GGT ATT TTT GTC CGA GTA GCA
TAT TAT GCA AAA TGG ATA CAC AAA ATT ATT
TTA ACA TAT AAG GTA CCA CAG TCA TAG

9. The gene coding for hepatic parenchymal cell growth factor, which is represented by the following base sequence extending from the 88th guanine to the last guanine in the sequence defined in claim 8:

```
5                                     GAG
GGA CAA AGG AAA AGA AGA AAT ACA ATT CAT
10 GAA TTC AAA AAA TCA GCA AAG ACT ACC CTA
ATC AAA ATA GAT CCA GCA CTG AAG ATA AAA
15 ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT
GCT AAT AGA TGT ACT AGG AAT AAA GGA CTT
CCA TTC ACT TGC AAG GCT TTT GTT TTT GAT
20 AAA GCA AGA AAA CAA TGC CTC TGG TTC CCC
TTC AAT AGC ATG TCA AGT GGA GTG AAA AAA
25 GAA TTT GGC CAT GAA TTT GAC CTC TAT GAA
AAC AAA GAC TAC ATT AGA AAC TGC ATC ATT
30 GGT AAA GGA CGC ACG TAC AAG GGA ACA GTA
TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG
35 CCC TGG AGT TCC ATG ATA CCA CAC GAA CAC
AGC TTT TTG CCT TCG AGC TAT CGG GGT AAA
GAC CTA CAG GAA AAC TAC TGT CGA AAT CCT
40 CGA GGG GAA GAA GGG GGA CCC TGG TGT TTC
ACA AGC AAT CCA GAG GTA CGC TAC GAA GTC
45 TGT GAC ATT CCT CAG TGT TCA GAA GTT GAA
TGC ATG ACC TGC AAT GGG GAG AGT TAT CGA
50 GGT CTC ATG GAT CAT ACA GAA TCA GGC AAG
ATT TGT CAG CGC TGG GAT CAT CAG ACA CCA
```

55

CAC CGG CAC AAA TTC TTG CCT GAA AGA TAT
 CCC GAC AAG GGC TTT GAT GAT AAT TAT TGC
 5 CGC AAT CCC GAT GGC CAG CCG AGG CCA TGG
 TGC TAT ACT CTT GAC CCT CAC ACC CGC TGG
 10 GAG TAC TGT GCA ATT AAA ACA TGC GGT GAC
 AAT ACT ATG AAT GAC ACT GAT GTT CCT TTG
 15 GAA ACA ACT GAA TGC ATC CAA GGT CAA GGA
 GAA GGC TAC AGG GGC ACT GTC AAT ACC ATT
 TGG AAT GGA ATT CCA TGT CAG CGT TGG GAT
 20 TCT CAG TAT CCT CAC GAG CAT GAC ATG ACT
 CCT GAA AAT TTC AAG TGC AAG GAC CTA CGA
 25 GAA AAT TAC TGC CGA AAT CCA GAT GGG TCT
 GAA TCA CCC TGG TGT TTT ACC ACT GAT CCA
 30 AAC ATC CGA GTT GGC TAC TGC TCC CAA ATT
 CCA AAC TGT GAT ATG TCA CAT GGA CAA GAT
 TGT TAT CGT GGG AAT GGC AAA AAT TAT ATG
 35 GGC AAC TTA TCC CAA ACA AGA TCT GGA CTA
 ACA TGT TCA ATG TGG GAC AAG AAC ATG GAA
 40 GAC TTA CAT CGT CAT ATC TTC TGG GAA CCA
 GAT GCA AGT AAG CTG AAT GAG AAT TAC TGC
 45 CGA AAT CCA GAT GAT GAT GCT CAT GGA CCC
 TGG TGC TAC ACG GGA AAT CCA CTC ATT CCT
 TGG GAT TAT TGC CCT ATT TCT CGT TGT GAA
 50 GGT GAT ACC ACA CCT ACA ATA GTC AAT TTA
 GAC CAT CCC GTA ATA TCT TGT GCC AAA ACG
 55 AAA CAA TTG CGA GTT GTA AAT GGG ATT CCA

ACA CGA ACA AAC ATA CGA TGG ATG GTT AGT
 5 TTG AGA TAC AGA AAT AAA CAT ATC TGC GGA
 GGA TCA TTG ATA AAG GAG AGT TGG GTT CTT
 10 ACT GCA CGA CAG TGT TTC CCT TCT CGA GAC
 TTG AAA GAT TAT GAA GCT TGG CTT GGA ATT
 15 CAT GAT GTC CAC GGA AGA GGA GAT GAG AAA
 TGC AAA CAG GTT CTC AAT GTT TCC CAG CTG
 GTA TAT GGC CCT GAA GGA TCA GAT CTG GTT
 20 TTA ATG AAG CTT GCC AGG CCT GCT GTC CTG
 GAT GAT TTT GTT AGT ACG ATT GAT TTA CCT
 25 AAT TAT GGA TGC ACA ATT CCT GAA AAG ACC
 AGT AGC AGT GTT TAT GGC TGG GGC TAC ACT
 30 GGA TTG ATC AAC TAT GAT GGC CTA TTA CGA
 GTG GCA CAT CTC TAT ATA ATG GGA AAT GAG
 35 AAA TGC AGC CAG CAT CAT CGA GGG AAG GTG
 ACT CTG AAT GAG TCT GAA ATA TGT GCT GGG
 GCT GAA AAG ATT GGA TCA GGA CCA TGT GAG
 40 GGG GAT TAT GGT GGC CCA CTT GTT TGT GAG
 CAA CAT AAA ATG AGA ATG GTT CTT GGT GTC
 45 ATT GTT CCT GGT CGT GGA TGT GCC ATT CCA
 AAT CGT CCT GGT ATT TTT GTC CGA GTA GCA
 50 TAT TAT GCA AAA TGG ATA CAC AAA ATT ATT
 TTA ACA TAT AAG GTA CCA CAG TCA TAG
 55

10. The gene coding for hepatic parenchymal cell growth factor, which is represented by the following base sequence extending from the 94th cytosine to the last guanine in the sequence defined in claim 8:

CAA AGG AAA AGA AGA AAT ACA ATT CAT
GAA TTC AAA AAA TCA GCA AAG ACT ACC CTA
5 ATC AAA ATA GAT CCA GCA CTG AAG ATA AAA
ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT
10 GCT AAT AGA TGT ACT AGG AAT AAA GGA CTT
CCA TTC ACT TGC AAG GCT TTT GTT TTT GAT
15 AAA GCA AGA AAA CAA TGC CTC TGG TTC CCC
TTC AAT AGC ATG TCA AGT GGA GTG AAA AAA
GAA TTT GGC CAT GAA TTT GAC CTC TAT GAA
20 AAC AAA GAC TAC ATT AGA AAC TGC ATC ATT
GGT AAA GGA CGC ACG TAC AAG GGA ACA GTA
25 TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG
CCC TGG AGT TCC ATG ATA CCA CAC GAA CAC
30 AGC TTT TTG CCT TCG AGC TAT CGG GGT AAA
GAC CTA CAG GAA AAC TAC TGT CGA AAT CCT
35 CGA GGG GAA GAA GGG GGA CCC TGG TGT TTC
ACA AGC AAT CCA GAG GTA CGC TAC GAA GTC
40 TGT GAC ATT CCT CAG TGT TCA GAA GTT GAA
TGC ATG ACC TGC AAT GGG GAG AGT TAT CGA
45 GGT CTC ATG GAT CAT ACA GAA TCA GGC AAG
ATT TGT CAG CGC TGG GAT CAT CAG ACA CCA

50

55

CAC CGG CAG AAA TTC TTG COT GAA AGA TAT
 CCC GAC AAG GGC TTT GAT GAT AAT TAT TGC
 5 CGG AAT CCC GAT GGC CAG CCG AGG CCA TGG
 TGC TAT ACT CTT GAC COT CAC ACC CGC TGG
 10 GAG TAC TGT GCA ATT AAA ACA TGC COT GAC
 AAT ACT ATG AAT GAC ACT GAT GTT COT TTG
 15 GAA ACA ACT GAA TGC ATC CAA GGT CAA GGA
 GAA GGC TAC AGG GGC ACT GTC AAT ACC ATT
 20 TGG AAT GGA ATT CCA TGT CAG CGT TGG GAT
 TCT CAG TAT CCT CAC GAG CAT GAC ATG ACT
 CCT GAA AAT TTC AAG TGC AAG GAC CTA CGA
 25 GAA AAT TAC TGC CGA AAT CCA GAT GGG TCT
 GAA TCA CCC TGG TGT TTT ACC ACT GAT CCA
 30 AAC ATC CGA GTT GGC TAC TGC TCC CAA ATT
 CCA AAC TGT GAT ATG TCA CAT GGA CAA GAT
 35 TGT TAT CGT GGG AAT GGC AAA AAT TAT ATG
 GGC AAC TTA TCC CAA ACA AGA TCT GGA CTA
 40 ACA TGT TCA ATG TGG GAC AAG AAC ATG GAA
 GAC TTA CAT CGT CAT ATC TTC TGG GAA CCA
 GAT GCA AGT AAG CTG AAT GAG AAT TAC TGC
 45 CGA AAT CCA GAT GAT GAT GCT CAT GGA CCC
 TGG TGC TAC ACG GGA AAT CCA CTC ATT CCT
 50 TGG GAT TAT TGC CCT ATT TCT CGT TGT GAA
 GGT GAT ACC ACA CCT ACA ATA GTC AAT TTA
 55 GAC CAT CCC GTA ATA TCT TGT GCC AAA ACC

5 AAA CAA TTG CGA GTT GTA AAT GGG ATT CCA
ACA CGA ACA AAC ATA GGA TGG ATG GTT AGT
10 TTG AGA TAC AGA AAT AAA CAT ATC TGC GGA
GGA TCA TTG ATA AAG GAG AGT TGG GTT CTT
ACT GCA CGA CAG TGT TTC COT TCT CGA GAC
15 TTG AAA GAT TAT GAA GCT TGG CTT GGA ATT
CAT GAT GTC CAC GGA AGA GGA GAT GAG AAA
TGC AAA CAG GTT CTC AAT GTT TCC CAG CTG
20 GTA TAT GGC CCT GAA GGA TCA GAT CTG GTT
TTA ATG AAG CTT GCC AGG CCT GCT GTC CTG
25 GAT GAT TTT GTT AGT ACG ATT GAT TTA CCT
AAT TAT GGA TGC ACA ATT CCT GAA AAG ACC
30 AGT AGC AGT GTT TAT GGC TGG GGC TAC ACT
GGA TTG ATC AAC TAT GAT GGC CTA TTA CGA
35 GTG GCA CAT CTC TAT ATA ATG GGA AAT GAG
AAA TGC AGC CAG CAT CAT CGA GGG AAG GTG
40 ACT CTG AAT GAG TCT GAA ATA TGT GCT GGG
GCT GAA AAG ATT GGA TCA GGA CCA TGT GAG
45 GGG GAT TAT GGT GGC CCA CTT GTT TGT GAG
CAA CAT AAA ATG AGA ATG GTT CTT GGT GTC
ATT GTT CCT GGT CGT GGA TGT GCC ATT CCA
50 AAT CGT CCT GGT ATT TTT GTC CGA GTA GCA
TAT TAT GCA AAA TGG ATA CAC AAA ATT ATT
55 TTA ACA TAT AAG GTA CCA CAG TCA TAG

11. An expression vector which comprises a gene coding for human hepatic parenchymal cell growth factor represented by the amino acid sequence defined in claim 1.
12. An expression vector which comprises the gene coding for human hepatic parenchymal cell growth factor and represented by the base sequence defined in claim 3.
13. Expression plasmid pKCRHGF-2.
14. A process for producing human hepatic parenchymal cell growth factor, comprising transforming a host cell with the expression vector defined in claim 11, and culturing the resulting transformant.
15. The process according to claim 14, wherein the transformation procedures are repeated.
16. The process according to claim 14 or 15, wherein the host cell is an animal cell.
17. A process for producing human hepatic parenchymal cell growth factor, comprising transforming a host cell with the expression vector defined in claim 12, and culturing the resulting transformant.
18. The process according to claim 17, wherein the transformation procedures are repeated.
19. The process according to claim 17 or 18, wherein the host cell is an animal cell.
20. An animal cell which is transformed with the expression vector according to claim 11 or 12 or the plasmid according to claim 13 and produces human hepatic parenchymal cell growth factor.
21. An animal cell according to claim 20, which is obtained by the repeated transformation as defined in claim 15 or 18.
22. A pharmaceutical composition, characterized in that it comprises a hHGF according to any of claims 1 to 4 together with a pharmaceutically acceptable diluent or excipient.
23. The use of a hHGF according to any of claims 1 to 4 for the preparation of a pharmaceutical.

25

30

35

40

45

50

55

Fig. 1 a

Met	Trp	Val	Thr	Lys	Leu	Leu	Pro	Ala	Leu	10
Leu	Leu	Gln	His	Val	Leu	Leu	His	Leu	Leu	20
Leu	Leu	Pro	Ile	Ala	Ile	Pro	Tyr	Ala	Glu	30
Gly	Gln	Arg	Lys	Arg	Arg	Asn	Thr	Ile	His	40
Glu	Phe	Lys	Lys	Ser	Ala	Lys	Thr	Thr	Leu	50
Ile	Lys	Ile	Asp	Pro	Ala	Leu	Lys	Ile	Lys	60
Thr	Lys	Lys	Val	Asn	Thr	Ala	Asp	Gln	Cys	70
Ala	Asn	Arg	Cys	Thr	Arg	Asn	Lys	Gly	Leu	80
Pro	Phe	Thr	Cys	Lys	Ala	Phe	Val	Phe	Asp	90
Lys	Ala	Arg	Lys	Gln	Cys	Leu	Trp	Phe	Pro	100
Phe	Asn	Ser	Met	Ser	Ser	Gly	Val	Lys	Lys	110
Glu	Phe	Gly	His	Glu	Phe	Asp	Leu	Tyr	Glu	120
Asn	Lys	Asp	Tyr	Ile	Arg	Asn	Cys	Ile	Ile	130
Gly	Lys	Gly	Arg	Ser	Tyr	Lys	Gly	Thr	Val	140
Ser	Ile	Thr	Lys	Ser	Gly	Ile	Lys	Cys	Gln	150
Pro	Trp	Ser	Ser	Met	Ile	Pro	His	Glu	His	160
Ser	Phe	Leu	Pro	Ser	Ser	Tyr	Arg	Gly	Lys	170
Asp	Leu	Gln	Glu	Asn	Tyr	Cys	Arg	Asn	Pro	180
Arg	Gly	Glu	Glu	Gly	Gly	Pro	Trp	Cys	Phe	190

Fig. 1 b

Thr	Ser	Asn	Pro	Glu	Val	Arg	Tyr	Glu	Val	200
Cys	Asp	Ile	Pro	Gln	Cys	Ser	Glu	Val	Glu	210
Cys	Met	Thr	Cys	Asn	Gly	Glu	Ser	Tyr	Arg	220
Gly	Leu	Met	Asp	His	Thr	Glu	Ser	Gly	Lys	230
Ile	Cys	Gln	Arg	Trp	Asp	His	Gln	Thr	Pro	240
His	Arg	His	Lys	<u>Phe</u>	<u>Leu</u>	<u>Pro</u>	<u>Glu</u>	<u>Arg</u>	<u>Tyr</u>	250
<u>Pro</u>	<u>Asp</u>	<u>Lys</u>	Gly	Phe	Asp	Asp	Asn	Tyr	Cys	260
Arg	Asn	Pro	Asp	Gly	Gln	Pro	Arg	Pro	Trp	270
Cys	Tyr	Thr	Leu	Asp	Pro	His	Thr	Arg	Trp	280
Glu	Tyr	Cys	Ala	Tyr	Lys	Thr	Cys	Ala	Asp	290
Asn	Thr	Met	Asn	Asp	Thr	Asp	Val	Pro	Leu	300
Glu	Thr	Thr	Glu	Cys	Ile	Gln	Gly	Gln	Gly	310
Ile	Gly	Tyr	Arg	Gly	Thr	Val	Asn	Thr	Ile	320
Trp	Asn	Gly	Ile	Pro	Cys	Gln	Arg	Trp	Asp	330
Ser	Gln	Tyr	Pro	His	Glu	His	Asp	Met	Thr	340
Pro	Glu	Asn	Phe	Lys	Cys	Lys	Asp	Leu	Arg	350
Glu	Asn	Tyr	Cys	Arg	Asn	Pro	Asp	Gly	Ser	360
Glu	Ser	Pro	Trp	Cys	Phe	Thr	Thr	Asp	Pro	370
Asn	Ile	Arg	Val	Gly	Tyr	Cys	Ser	Gln	Ile	380

Fig. 1 c

Pro	Asn	Cys	Asp	Met	Ser	His	Gly	Gln	Asp	390
Cys	Tyr	Arg	Gly	Asn	Gly	Lys	Asn	Tyr	Met	400
Gly	Asn	Leu	Ser	Gln	Thr	Arg	Ser	Gly	Leu	410
Thr	Cys	Ser	Met	Trp	Asp	Lys	<u>Asn</u>	<u>Met</u>	<u>Glu</u>	420
<u>Asp</u>	<u>Leu</u>	<u>His</u>	<u>Arg</u>	<u>His</u>	<u>Ile</u>	<u>Phe</u>	<u>Trp</u>	<u>Glu</u>	<u>Pro</u>	430
<u>Asp</u>	<u>Ala</u>	<u>Ser</u>	<u>Lys</u>	Leu	Asn	Glu	Asn	Tyr	Cys	440
Arg	Asn	Pro	Asp	Asp	Asp	Ala	His	Gly	Pro	450
Trp	Cys	Tyr	Thr	Gly	Asn	Pro	Leu	Ile	Pro	460
Trp	Asp	Tyr	Cys	Pro	Ile	Ser	Arg	Cys	Glu	470
Gly	Asp	Thr	Thr	Pro	Thr	Ile	Val	Asn	Leu	480
Asp	His	Pro	Val	Ile	Ser	Cys	Ala	Lys	Thr	490
Lys	Gln	Leu	Arg	Val	Val	Asn	Gly	Ile	Pro	500
Thr	Arg	Thr	Asn	Ile	Gly	Trp	Met	Val	Ser	510
Leu	Arg	Tyr	Arg	Asn	Lys	His	Ile	Cys	Gly	520
Gly	Ser	Leu	Ile	Lys	Glu	Ser	Trp	Val	Leu	530
Thr	Ala	Arg	Gln	Cys	Phe	Pro	Ser	Arg	Asp	540
Leu	Lys	<u>Asp</u>	<u>Tyr</u>	<u>Glu</u>	<u>Ala</u>	<u>Trp</u>	<u>Leu</u>	<u>Gly</u>	<u>Ile</u>	550
<u>His</u>	<u>Asp</u>	<u>Val</u>	<u>His</u>	<u>Gly</u>	<u>Arg</u>	<u>Gly</u>	<u>Asp</u>	<u>Glu</u>	<u>Lys</u>	560
Cys	Lys	Gln	Val	Leu	Asn	Val	Ser	Gln	Leu	570

Fig. 1 d

Val Tyr Gly Pro Glu Gly Ser Asp Leu Val 580
 Leu Met Lys Leu Ala Arg Pro Ala Val 590
 Asp Asp Phe Val Ser Thr Ile Asp Leu 600
 Asn Tyr Gly Cys Thr Ile Pro Glu Lys 610
 Ser Cys Ser Val Tyr Gly Trp Gly Tyr 620
 Gly Leu Ile Asn Tyr Asp Gly Leu Leu 630
 Val Ala His Leu Tyr Ile Met Gly Asn 640
 Lys Cys Ser Gln His His Arg Gly Lys 650
 Thr Leu Asn Glu Ser Glu Ile Cys Ala 660
 Ala Glu Lys Ile Gly Ser Gly Pro Cys 670
 Gly Asp Tyr Gly Gly Pro Leu Val Cys 680
 Gln His Lys Met Arg Met Val Leu Gly 690
 Ile Val Pro Gly Arg Gly Cys Ala Ile 700
 Asn Arg Pro Gly Ile Phe Val Arg Val 710
 Tyr Tyr Ala Lys Trp Ile His Lys Ile 720
 Leu Thr Tyr Lys Val Pro Gln Ser

Fig. 2 a

	60
ATG TGG GTG ACC AAA CTC CTG CCA GCC CTG CTG Pst I CAT GTC CTC CTG CAT CTC CTC	
CTG CTC CCC ATC GCC ATC CCC TAT GCA GAG GGA CAA AGG AAA AGA AAT ACA ATT CAT	120
GAA TTC AAA AAA TCA GCA AAG ACT ACC CTA ATC AAA ATA GAT CCA GCA CTG AAG ATA AAA Eco RI	180
ACC AAA AAA GTG AAT ACT GCA GAC CAA TGT GCT AAT AGA TGT ACT AGG AAT AAA GGA CTT	240
CCA TTC ACT TGC AAG GCT TTT GTT TTT GAT AAA GCA AGA AAA CAA TGC CTC TGG TTC CCC	300
TTC AAT AGC ATG TCA AGT GGA GTG AAA AAA GAA TTT GGC CAT GAA TTT GAC CTC TAT GAA	360
AAC AAA GAC TAC ATT AGA AAC TGC ATC ATT GGT AAA GGA CGC AGC TAC AAG GGA ACA GTA	420
TCT ATC ACT AAG AGT GGC ATC AAA TGT CAG CCC TGG AGT TCC ATG ATA CCA CAC GAA CAC	480
AGC TTT TTG CCT TCG AGC TAT CGG GGT AAA GAC CTA CAG GAA AAC TAC TGT CGA AAT CCT Xho I	540
CGA GGG GAA GAA GGG GGA CCC TGG TGT TTC ACA AGC AAT CCA GAG GTA CGC TAC GAA GTC	600
TGT GAC ATT CCT CAG TGT TCA GAA GTT GAA TGC ATG ACC TGC AAT GGG GAG AGT TAT CGA	660
GGT CTC ATG GAT CAT ACA GAA TCA GGC AAG ATT TGT CAG CGC TGG GAT CAT CAG ACA CCA	720
CAC CGG CAC AAA TTC TTG CCT GAA AGA TAT CCC GAC AAG GGC TTT GAT GAT AAT TAT TGC	780

Fig. 2 c

```

TTG AAA GAT TAT GAA GCT TGG CTT GGA ATT CAT GAT GTC CAC GGA AGA GGA GAT GAG AAA 1680
      Eco RI
TGC AAA CAG GTT CTC AAT GTT TCC CAG CTG GTA TAT GGC CCT GAA GGA TCA GAT CTG GTT 1740
TTA ATG AAG CTT GCC AGG CCT GCT GTC CTG GAT GAT TTT GTT AGT ACG ATT GAT TTA CCT 1800
AAT TAT GGA TGC ACA ATT CCT GAA AAG ACC AGT TGC AGT GTT TAT GGC TGG GGC TAC ACT 1860
GGA TTG ATC AAC TAT GAT GGC CTA TTA CGA GTG GCA CAT CTC TAT ATA ATG GGA AAT GAG 1920
AAA TGC AGC CAG CAT CAT CGA GGG AAG GTG ACT CTG AAT GAG TCT GAA ATA TGT GCT GGG 1980
GCT GAA AAG ATT GGA TCA GGA CCA TGT GAG GGG GAT TAT GGT GGC CCA CTT GTT TGT GAG 2040
CAA CAT AAA ATG AGA ATG GTT CTT GGT GTC ATT GTT CCT GGT GGA TGT GCC ATT CCA 2100
AAT CGT CCT GGT ATT TTT GTC CGA GTA GCA TAT TAT GCA AAA TGG ATA CAC AAA ATT ATT 2160

TTA ACA TAT AAG GTA CCA CAG TCA TAG
      Kpn I

```

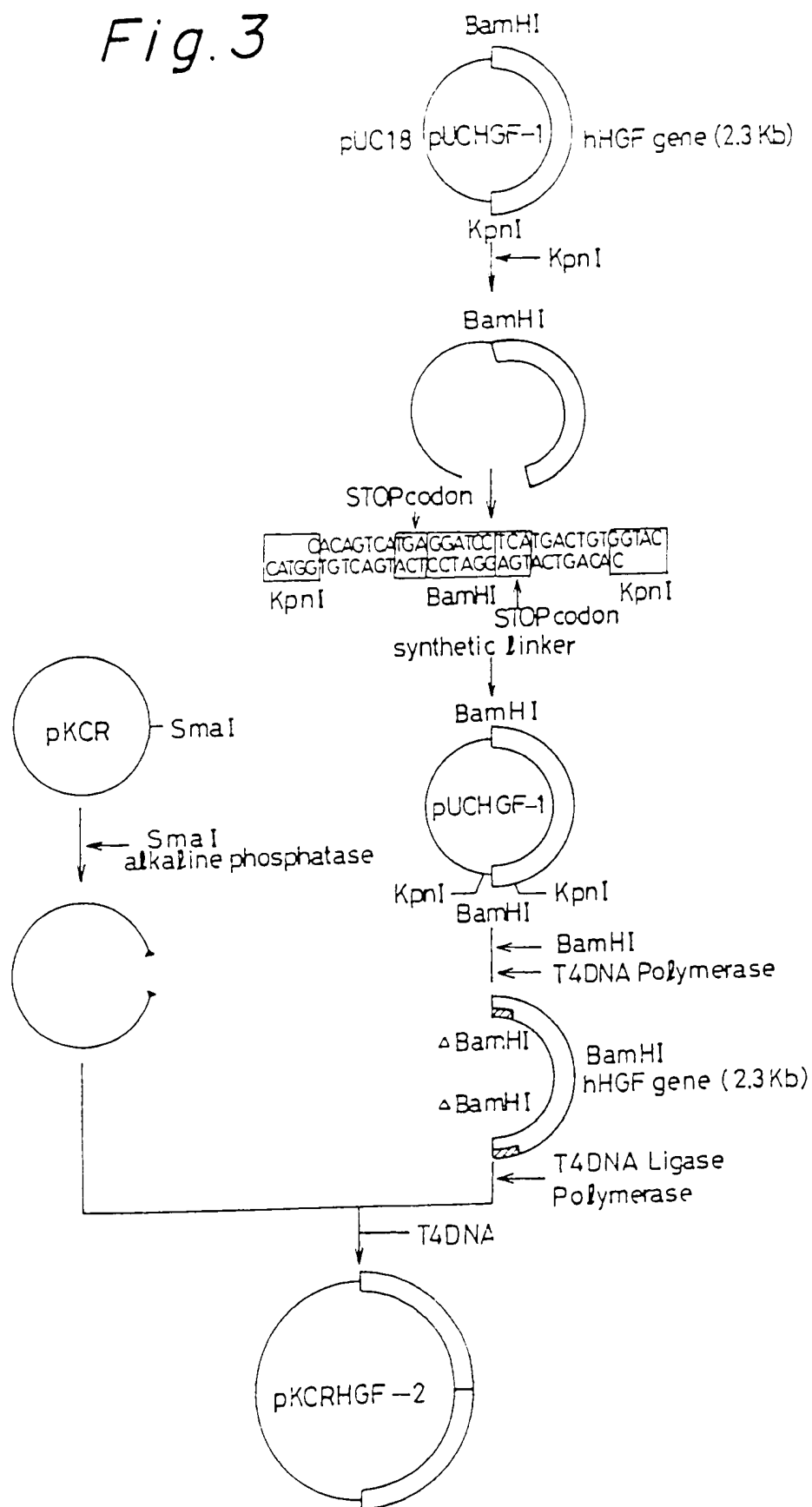
Fig. 3

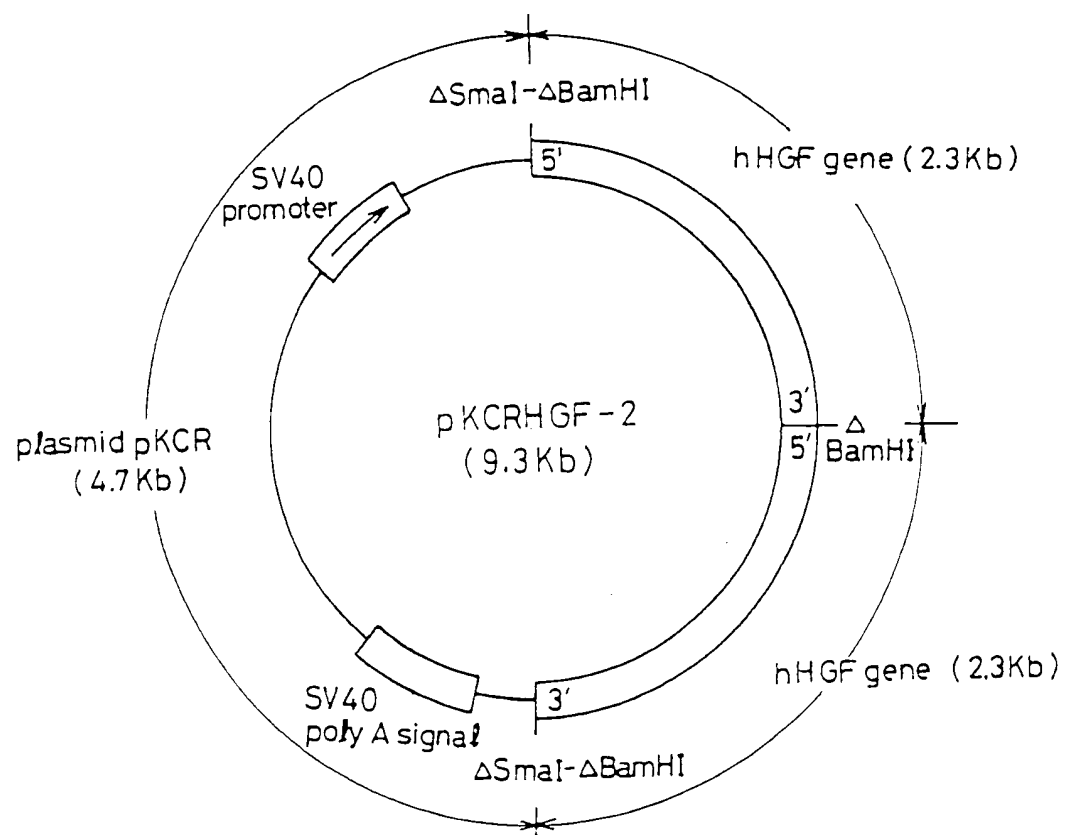
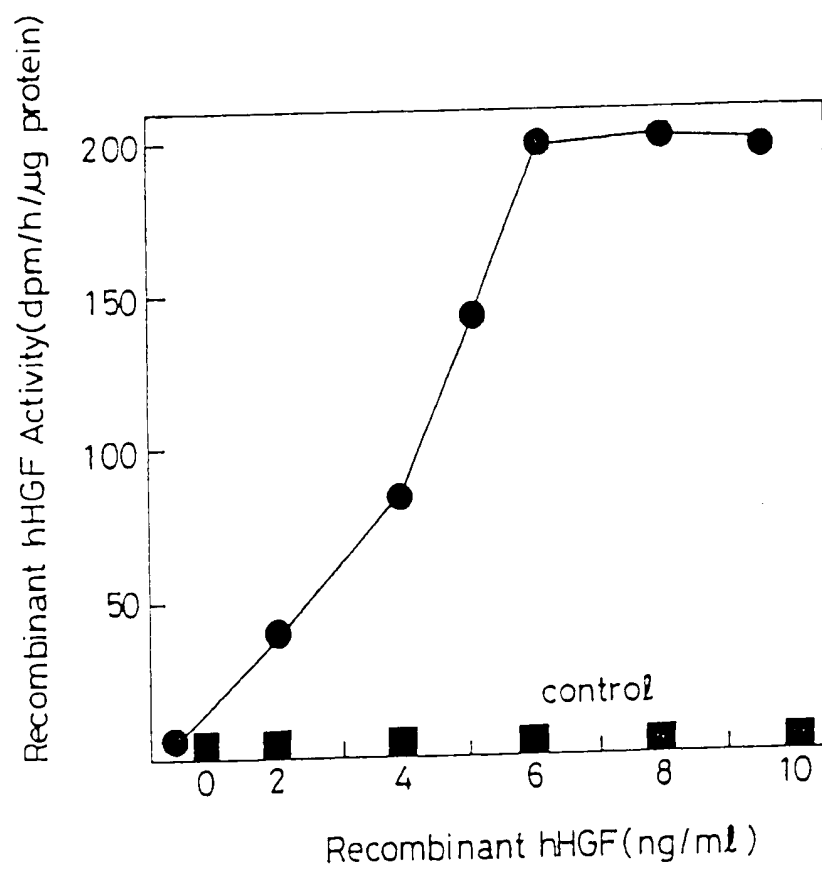
Fig. 4

Fig. 5



European
Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 11 5397

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P,X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 183, no. 2, 15th September 1989, pages 967-973, Academic Press, Inc.; K. MIYAZAWA et al.: "Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor" * Whole document *	1-12	C 07 K 13:00 C 12 N 15:16 C 12 P 21:02 C 12 N 5:10 A 61 K 37:24
P,X	NATURE, vol. 342, 23rd November 1989, pages 440-443; T. NAKAMURA et al.: "Molecular cloning and expression of human hepatocyte growth factor" * Whole document *	1-12, 22-23	
X	CANCER RESEARCH, vol. 49, 15th June 1989, pages 3314-3320; R. Zarnegar et al.: "Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes" * Whole document *	1-4	
X	J. CLIN. INVEST., vol. 81, February 1988, pages 414-419; E. GOHDA et al.: "Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure" * Whole document *	1-4	
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 12 P C 12 N C 07 K
Place of search		Date of completion of search	Examiner
The Hague		16 November 90	NAUCHE S.A.
CATEGORY OF CITED DOCUMENTS			E: earlier patent document, but published on, or after the filing date O: document cited in the application L: document cited for other reasons
X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention			S: member of the same patent family, corresponding document